Journal of Forensic and Legal Medicine 20 (2013) 143–145

Contents lists available at SciVerse ScienceDirect

Journal of Forensic and Legal Medicine

journal homepage: www.elsevier.com/locate/jflm

Original communication

Cocaine postmortem distribution in three brain structures: A comparison with whole blood and vitreous humour

Virginia M. Carvalho PhD [a,*], André R. Fukushima MD [a], Luiz R. Fontes PhD [b], Daniela V. Fuzinato MD [b], Jorge C. Florio PhD [c], Alice A.M. Chasin PhD [a]

[a] Universidade de São Paulo, Faculdade de Ciências Farmacêuticas, Departamento de Análises Clínicas e Toxicológicas, Av. Prof. Lineu Prestes, 580, 05508-900 São Paulo, Brazil
[b] Instituto Médico Legal de São Paulo, Av. Dr. Enéas de Carvalho Aguiar, 600, 05403-000 São Paulo, Brazil
[c] Universidade de São Paulo, Faculdade de Medicina Veterinária e Zootecnia, Departamento de Patologia, Av. Prof. Dr. Orlando Paiva, 87, 05508-270 São Paulo, Brazil

ARTICLE INFO

Article history:
Received 16 January 2012
Received in revised form
6 June 2012
Accepted 17 June 2012
Available online 12 July 2012

Keywords:
Cocaine
Postmortem
Brain
Vitreous humour
Whole blood

ABSTRACT

The presence of cocaine (COC) in fluids or tissues does not prove that death was due to drug consumption and the interpretation of postmortem concentrations is more complex than attempts at making such correlations in the living. The purpose of this study was to investigate the distribution of cocaine and its metabolite benzoylecgonine in brain and compare with whole blood and vitreous humour. The distribution in three brain structures (prefrontal cortex, basal ganglia and cerebellum) was homogeneous. There is a strong correlation for cocaine concentrations between vitreous humour and brain, vitreous humour and whole blood, and whole blood and brain in overdose cases. In addition, the comparison of COC/benzoylecgonine (BE) ratios in different experimental specimens proved to be more appropriate for evaluating cocaine-related death than individual drug values. These findings suggest that the comparison of cocaine levels in different compartments is essential to assess the cause of death.

1. Introduction

Cocaine (COC) is one of the most frequent causes of drug-related death reported by forensic pathologists. The diagnostic criteria for a death from acute COC intoxication commonly include a review of police investigation, autopsy, identification and quantification of COC and its metabolites in the blood.[1,2] However, the postmortem redistribution hinders the interpretation of blood levels of cocaine[3] and the analysis of other body elements could improve the diagnosis of cocaine-related death.

Brain tissue (BR) is a potentially valuable specimen, since: (1) its metabolic activity is low, resulting in a slower COC degradation, (2) the metabolite benzoylecgonine (BE) does not cross the blood–brain barrier and (3) it is inside an isolated compartment, where the drug concentrations measured are close or equal to perimortem concentrations.[4,5] Besides, there is the possibility, already demonstrated by other authors, of establishing the occurrence of death from overdose through studies of the distribution and concentration ratios of COC/BE in brain and blood.[6] In addition, vitreous humour (VH) is a better-preserved sample and is easier to collect. Thus, VH is a good alternative to whole blood (WB) analysis in determining the concentration of COC and BE.[7]

It is recommended that 50 g of BR be used for analysis[8]; however, no more than 10 g is typically needed. BR is a heterogeneous tissue weighing about 1000 g.

In order to determine COC and BE concentrations in BR, VH and WB, the samples from COC consumers were analysed.

2. Methods

WB from cardiac chamber, VH, and BR tissue from prefrontal cortex (PC), basal ganglia (BG) and cerebellum (CB) were collected postmortem from 18 known COC consumers. Fifteen were male and three female, from 16 to 45 years with a mean age of 28 years. The WB specimens were preserved with sodium fluoride.

A prilocaine solution was used as internal standard (IS).

BR tissues (5 g) were homogenised in 20 ml of a phosphate buffer (0.1 M, pH 6.0); after the addition of IS (5 μg g^{-1}), the homogenate was centrifuged at 4000 rpm, 4 °C, for 70 min. VH samples (2.0 ml) were added to 5 ml of 0.1 M phosphate buffer (pH 6.0) and mixed with the IS (3.00 μg ml^{-1}). Deproteinised ($ZnSO_4$ 5%) WB samples (3.0 ml) were mixed with the IS (3.00 μg ml^{-1}).

* Corresponding author. Calle 58 No. 20-45, Bogota D.C., Colombia. Tel.: +57 1 8108190.
E-mail address: vmcar@usp.br (V.M. Carvalho).

1752-928X/$ – see front matter © 2012 Elsevier Ltd and Faculty of Forensic and Legal Medicine. All rights reserved.
http://dx.doi.org/10.1016/j.jflm.2012.06.006

The prepared samples were extracted by SPE with 'Bond Elut Certify I' column from Varian according to manufacturers' specifications and the analytes quantified in an Agilent HPLC-DAD Model 1100 Series with isocratic mode and reversed phase, UV detection at 235 nm, mobile phase 0.025 M phosphate buffer pH 6.00 and acetonitrile (80:20 v/v), flow 1 ml min l^{-1} and injection volume of 100 μl.

The software InStat3 was used in statistical analysis (p-value >0.05 was considered not significant and $r \geq 0.75$ was considered strong in correlation tests).

3. Results and discussion

The analyte concentrations are summarised in Table 1. The histories related with the death include recreational use, heavy use and a body packing case.

The median comparison by variance analysis (ANOVA) showed a homogeneous distribution in the three brain segments with p-value = 0.7350 and p-value = 0.5428 for COC and BE concentrations, respectively, and any of its structures could be analysed in the COC use evaluation. Although the brain tissue preparations need more time than WB and VH (70 min in refrigerate centrifuge), the COC and BE concentrations in brain tissue are useful to estimating the disposition of COC and its biotransformation product, BE, at collection time and consequently at the time of death since the postmortem redistribution, although occurs, is time-dependent and the relationship (ratio) is maintained.

There have been many *in vivo* studies performed to determine COC blood concentrations and these data are used when performing postmortem analysis. On the other hand, many factors may lead to problems in interpreting the concentrations, such as the collect compartment. The peripheral blood collection is recommended for quantitative analysis, although, inevitably, there will be situations in which such sample collection is not possible.[9] In this study, blood from cardiac chamber was used because in many cases the femoral vessels were colabated and peripheral blood collection was impossible.

Generally, the blood levels should be compared with other compartments, such as VH and BR. The VH has become an alternative specimen in cases where WB samples were not available or suitable due to severe trauma or exsanguinations,[10] and the collection and preparation are fast and simple. In addition, the brain drug concentrations are close to the levels in the moment immediately before death because it is an isolated compartment with low enzymatic activity. The BE does not cross the blood–brain barrier by the high polarity and the levels showed in this matrix were formed in locus.

In order to verify a relation between the matrix and the COC and BE levels in population, the COC/BE ratios were calculated and the cases were categorised in overdose or incidental by the autopsy data and death circumstance (Table 2). Bertol et al. (2008) and Spiehler and Reed (1985) showed higher COC/BE ratio means and higher COC concentration means for BR/WB ratio in overdose cases. In the present study, although the COC/BE means are higher in most of the overdose cases, no difference by unpaired t-test was demonstrated in BR (p-value = 0.7360), WB (p-value = 0.0589) and VH (0.0750). Similarly, no differences were shown between the two categories for BR/WB ratio (p-value = 0.7874 for COC and p-value = 0.3968 for BE), BR/HV ratio (p-value = 0.8383 for COC and p-value = 0.3340 for BE) and WB/HV ratio (p-value = 0.6273 for COC and p-value = 0.8263 for BE) by unpaired t-test for means comparison (Table 2). On the other hand, in most of the overdose cases the COC/BE ratio was higher than 1. The percentage of COC/BE >1.0 was 57%, 100% and 29% for overdose and 18%, 73% and 0% for incidental cases in HV, BR and WB, respectively.

These results demonstrate that the COC-related cases are very complex and factors such as individual susceptibility, chronic and recreational use represent confusion variables and render difficult establishing a standard.

However, it was found that VH has lower analyte concentrations than WB[11,12] and the VH shows higher COC/BE ratios than WB. This could be justified by the lower enzymatic activity than blood[10] and, consequently, low COC degradation. Although the VH samples show alkaline pH (mean = 7.8, SD ± 0.73) related to spontaneous hydrolysis and the postmortem dehydration of the vitreous chamber can be related to the increase in analyte concentrations, the VH humour could be a valuable sample because the postmortem redistribution is slower[3] and the analytical preparation is easier than WB. On the other hand, it is necessary to determine the

Table 1
Cocaine and benzoylecgonine concentrations in samples.

Case	History	BR-PC [μg/g]		BR-BG [μg/g]		BR-CB [μg/g]		WB [μg/mL]		VH [μg/mL]		COC/BE ratio		
		COC	BE	COC	BE	COC	BE	COC	BE	COC	BE	BR-BG	WB	VH
A	Sudden death	0.61	0.24	0.82	0.33	0.52	0.25	0.11	3.26	0.58	1.07	2.48	0.03	0.54
B	Sudden death	0.64	0.26	0.77	0.32	0.82	0.36	0.63	0.28	1.56	0.35	2.41	2.25	4.46
C	Sudden death	0.30	0.26	0.47	ND	0.57	0.26	0.31	0.97	0.59	1.04	2.35	0.32	0.57
E	Sudden death	0.10	ND	0.26	ND	ND	ND	1.04	0.63	0.12	0.21	1.30	1.65	0.57
H	Sudden death	0.46	0.30	0.64	0.31	0.58	0.27	ND	0.89	0.50	0.37	2.06	0.10	1.35
Q	Sudden death	19.61	3.21	17.61	2.90	24.44	5.84	13.15	15.03	4.66	1.28	6.07	0.87	3.64
F	To be run over	0.85	0.25	1.00	0.32	0.82	0.25	ND	0.68	0.64	1.19	3.13	0.13	0.54
L	To be run over	1.79	1.59	2.71	1.60	2.43	1.69	1.62	4.43	1.26	3.19	1.69	0.37	0.39
K	Precipitation	0.11	0.63	0.16	0.60	0.21	0.85	ND	0.48	ND	0.55	0.27	0.19	0.16
D	Precipitation	0.25	0.21	0.49	0.29	0.39	0.34	ND	0.54	0.48	1.36	1.69	0.17	0.35
O	Precipitation	5.55	0.71	3.97	0.36	4.59	0.45	2.57	3.25	6.09	2.80	11.03	0.79	2.18
I	Homicide	0.36	0.54	0.50	0.95	0.53	0.89	ND	0.34	0.44	0.73	0.53	0.26	0.60
J	Homicide	0.22	0.32	0.28	0.22	0.31	0.37	ND	0.62	ND	0.71	1.27	0.15	0.13
G	Homicide	1.00	0.46	0.88	0.50	0.83	0.59	0.59	2.44	0.54	0.91	1.76	0.24	0.59
M	Homicide	0.25	0.93	0.47	1.98	0.45	2.48	0.83	7.21	0.36	1.39	0.24	0.12	0.26
N	Homicide	0.99	ND	1.20	ND	1.14	ND	0.30	0.38	1.10	0.41	6.00	0.79	2.68
P	COC chronic use	3.78	0.71	4.36	0.72	4.85	2.59	6.95	33.95	0.81	1.51	6.06	0.20	0.54
R	Body packing	12.56	2.38	23.43	2.81	13.71	2.47	24.43	34.74	10.97	5.91	8.34	0.70	1.86
	Mean	2.75	0.72	3.33	0.79	3.18	1.11	2.92	6.12	1.71	1.39	3.26	0.52	1.19
	Range	0.10	ND	0.16	ND	ND	ND	ND	0.28	ND	0.21	0.24	0.03	0.13
		19.61	3.21	23.43	2.90	24.44	5.84	24.43	34.74	10.97	5.91	11.03	2.25	4.46

ND-Not detected; COC-cocaine; BE-benzoylecgonine; BR-PC-brain-prefrontal cortex; BR-BG-brain-basal ganglia; BR-CB-brain cerebellum; WB-whole blood; VH-vitreous humour.

Table 2
Analytes comparison in the matrix.

		Bertol et al., 2008				Spiehler and Reed, 1985	
		Fatalities		Fatalities		Fatalities	
		Overdose ($N = 7$)	Incidental ($N = 11$)	Overdose ($N = 84$)	Incidental ($N = 33$)	Overdose ($N = 37$)	Incidental ($N = 46$)
COC/BE ratio mean ± S.D.							
VH		1.86 ± 1.59	0.77 ± 0.85				
BR-BG		3.57 ± 2.60	3.06 ± 3.35	10.28 ± 5.79	0.71 ± 0.61	14.70	0.87
WB		0.85 ± 0.83	0.31 ± 0.25	0.69 ± 0.53	0.21 ± 0.15	0.64	0.27
Matrix ratios mean ± S.D.							
BR/HV	COC	1.72 ± 1.10	1.85 ± 1.31				
	BE	0.89 ± 0.70	0.62 ± 0.45				
WB/HV	COC	2.15 ± 3.06	1.50 ± 2.43				
	BE	3.97 ± 3.82	3.36 ± 6.49				
BR/WB	COC	2.73 ± 2.91	3.11 ± 2.78	8.06 ± 4.45	2.28 ± 1.23	9.60	0.36
	BE	0.36 ± 0.36	0.64 ± 0.79	0.67 ± 0.47	1.67 ± 0.93	2.50	1.40

Table 3
R values obtained by the Pearson correlation tests.

Matrix	Fatalities			
	Overdose		Incidental	
	COC	BE	COC	BE
VH × WB	0.9843	0.9525	0.2845	0.1869
WB × BR	0.9831	0.8858	0.8657	0.1896
VH × BR	0.9476	0.7126	0.6569	0.3845

relation with the WB because this matrix is used as reference for *in vivo* and postmortem cases.

The compartments were compared by the Pearson's correlation tests and Table 3 shows the *r*-values. In overdose cases, the COC concentrations showed strong correlation between VH × WB, WB × BR and VH × BR. On the other hand, in incidental cases strong correlation was demonstrated for COC concentrations only between WB × BR. The BE concentrations show strong correlation between VH × WB and WB × BR. Many authors demonstrated a strong correlation between VH and WB for COC and BE concentrations.[11,13] These results show that it is possible to use a linear regression model to infer the COC and BE concentrations in WB using VH, and COC concentrations in BR using VH or WB in overdose cases, but not in incidental cases. In addition, the results suggest that VH is useful to infer the COC concentrations in overdose cases. Besides, the VH was more adequate to identify the COC use than WB; COC was not detected in WB of six cases against two cases in VH (Table 1). Although more studies are recommended to clarify this point, it means that VH supplies information that is qualitative (to determine the COC use) and quantitative (to estimate the blood COC concentrations and determine an overdose case).

The COC/BE ratio could be used to determine if COC use was recent, or if a chronic or recreational exposure has occurred and, in both cases, COC, although present, would not be considered the primary agent of mortality.

In case E (Table 1), the brain tissue and vitreous humour COC concentrations were low, but the circumstances, autopsy and WB concentration suggest a classical overdose with COC/BE ratio >1. In case L, all specimens have increased analyte contents and the WB COC concentrations suggest a classical overdose, but the COC/BE ratios were <1 for WB and VH and the cause of death was polytrauma as a result of being run over. COC could be the reason for a person being subjected to the trauma, for example, an agitated delirious person running in front a car[14] and for evaluating this situation the brain concentration could be valuable information (COC/BE ratio was >1 in brain). Also, the COC/BE ratios were more useful for evaluating the individual cases than the isolate concentrations and it is valuable to infer an overdose case.

Although the BR is the most useful matrix for postmortem COC-related cases, the VH could be a gold standard in cases where analysis of different samples is unviable.

Ethical approval
None declared.

Funding
None declared.

Conflict of interest
None declared.

References

1. Darke S, Kaye S, Duflou J. Cocaine-related fatalities in New South Wales, Australia 1993–2002. *Drug Alcohol Depend* 2005;**77**:107–14.
2. Jenkins AJ, Levine B, Titus J, Smialek JE. The interpretation of cocaine and benzoylecgonine concentrations in postmortem cases. *Forensic Sci Int* 1999;**101**:17–25.
3. Alicot AP, Gaulier J, Champsaur P, Marquet P. Mechanisms underlying postmortem redistribution of drugs: a review. *J Anal Toxicol* 2003;**27**:533–44.
4. Stimpfl T, Reichel S. Distribution of drugs of abuse within specific regions of the human brain. *Forensic Sci. Int.* 2007;**170**:179–82.
5. Spiehler VR, Reed D. Brain concentrations of cocaine and benzoylecgonine in fatal cases. *J Forensic Sci* 1985;**30**:1003–11.
6. Bertol E, Trignano C, Di Milia MG, Di Padua M, Mari F. Cocaine-related deaths: an enigma still under investigation. *Forensic Sci Int* 2008;**176**:121–3.
7. Fernandez P, Rodriguez P, Bermejo AM, Lopez-Rivadulla M, Cruz A. Simultaneous determination of cocaine and benzoylecgonine in vitreous by HPLC. *J Liq Chromatogr* 1994;**17**(4):883–90.
8. SOFT/AAFS Forensic Laboratory Guidelines. Available from: http://www.soft-tox.org/files/Guidelines_2006_Final.pdf; 2006.
9. Cook DS, Braithwaite RA, Hale KA. Estimating antemortem drug concentrations from postmortem blood samples: the influence of postmortem redistribution. *J Clin Pathol* 2000;**53**:282–5.
10. Holmgren P, Druid H, Holmgren A, Ahlner J. Stability of drugs in stored postmortem femoral. *J Forensic Sci* 2004;**49**(4):1–6.
11. Mackey-Bojack S, Kloss J, Apple F. Cocaine, cocaine metabolite, and ethanol concentrations in postmortem blood and vitreous humor. *J Anal Toxicol* 2000;**24**(1):59–65.
12. Lucena J, Blanco M, Jurado C, Rico A, Salguero M, Vazquez R, et al. Cocaine-related sudden death: a prospective investigation in south-west Spain. *Eur Heart J* 2010. http://dx.doi.org/10.1093/eurheart/ehp557.
13. Duer WC, Spitz DJ, Macfarland S. Relationships between concentrations of cocaine and its hydrolysates in peripheral blood, heart blood, vitreous humor and urine. *J Forensic Sci* 2006;**51**(2):421–5.
14. Stephens BG, Jentzen JM, Karch S, Mash DC, Wetli CV. Criteria for the interpretation of cocaine levels in human biological samples and their relation to the cause of death. *Am J Forensic Med Pathol* 2004;**25**(1):1–10.